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Perfluoroalkyl Substances during Pregnancy and Offspring Weight and Adiposity at Birth: Examining Mediation by Maternal Fasting Glucose in the Healthy Start Study

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Background: Certain perfluoroalkyl and polyfluoroalkyl substances (PFAS) are widespread, persistent environmental contaminants. Prenatal PFAS exposure has been associated with lower birth weight; however, impacts on body composition and factors responsible for this association are unknown.

Objectives: We aimed to estimate associations between maternal PFAS concentrations and offspring weight and adiposity at birth, and secondarily to estimate associations between PFAS concentrations and maternal glucose and lipids, and to evaluate the potential for these nutrients to mediate associations between PFAS and neonatal outcomes.

Methods: Within the Healthy Start prospective cohort, concentrations of 11 PFAS, fasting glucose, and lipids were measured in maternal mid-pregnancy serum ($n = 628$). Infant body composition was measured using air displacement plethysmography. Associations between PFAS and birth weight and adiposity, and between PFAS and maternal glucose and lipids, were estimated via linear regression. Associations were decomposed into direct and indirect effects.

Results: Five PFAS were detectable in >50% of participants. Maternal perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) concentrations were inversely associated with birth weight. Adiposity at birth was approximately 10% lower in the highest categories of PFOA, PFNA, and perfluorohexane sulfonate (PFHxS) compared to the lowest categories. PFOA, PFNA, perfluorodecanoate (PFDeA), and PFHxS were inversely associated with maternal glucose. Up to 11.6% of the effect of PFAS on neonatal adiposity was mediated by maternal glucose concentrations. Perfluorooctane sulfonate (PFOS) was not significantly associated with any outcomes studied.

Conclusions: Follow-up of offspring will determine the potential long-term consequences of lower weight and adiposity at birth associated with prenatal PFAS exposure. https://doi.org/10.1289/EHP641

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are widespread and persistent environmental contaminants detected in many populations worldwide, including among pregnant women and infants (Cariou et al. 2015; Jiang et al. 2014; Kato et al. 2014; Manzano-Salgado et al. 2015; Okada et al. 2013). PFAS have been used for decades in industrial and commercial applications, including surface treatments for fabrics, food packaging, and aqueous film-forming foams for extinguishing fires (Buck et al. 2011; Prevedouros et al. 2006). The serum concentrations of certain PFAS have declined in the United States over the past decade (CDC 2015) following the phase out of perfluorooctane sulfonate (PFOS) production by 3M in 2000–2002 (U.S. EPA 2000) and the listing of PFOS in Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009 (Secretariat of the Stockholm Convention 2010). However, other PFAS concentrations have remained relatively constant over the same time period (CDC 2015), suggesting that human exposure is ongoing.

The ubiquitous presence of certain PFAS in humans is of concern because animal studies have demonstrated hepatotoxicity, immunotoxicity, and developmental toxicity resulting from high dose exposure (Lau et al. 2007). Epidemiologic studies in a population highly exposed to one PFAS, perfluorooctanoate (PFOA), have shown associations between PFAS exposure and chronic diseases, including kidney and testicular cancers (Barry et al. 2013), ulcerative colitis (Steenland et al. 2013), high cholesterol (Steenland et al. 2009), and pregnancy-induced hypertension (Darrow et al. 2013). Strong correlations between maternal and cord blood PFAS concentrations suggest transfer to the fetus from maternal circulation (Aylward et al. 2014). Maternal concentrations of PFOA during pregnancy have been previously associated with lower offspring birth weight in systematic reviews of the human evidence (Bach et al. 2015a; Johnson et al. 2014), as well an integrative assessment of human and nonhuman animal evidence (Lam et al. 2014), but the factors responsible for this association, if causal, have not been established. Moreover, some recent studies have reported positive associations between maternal PFAS concentrations during pregnancy and offspring body weight, waist circumference, and other indicators of adiposity in childhood and early adulthood (Braun et al. 2016; Halldorsson et al. 2012; Høyer et al. 2015; Mora et al. 2017), while another study reported null associations (Andersen et al. 2013).

Fetal growth depends on the transfer of nutrients, including glucose, amino acids, and free fatty acids, from the mother to the fetus (Jansson and Powell 2013). Excess maternal circulating nutrients, as in maternal obesity and gestational diabetes, have been associated with fetal overgrowth (macrosomia) and greater adiposity at birth (Catalano et al. 2003; Freinkel

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1980). Overnutrition and undernutrition *in utero* have both been associated with greater risk of obesity and metabolic disease in adulthood (Baird et al. 2005; Claris et al. 2010). Adiposity at birth may be a stronger predictor of future obesity risk than birth weight alone (Catalano et al. 2009). Therefore, it is important to examine whether environmental exposures influence fetal growth, and, in particular, fat mass accretion, with the goal of preventing long-term chronic disease. To our knowledge, no previous studies have examined the association between maternal PFAS concentrations during pregnancy and offspring adiposity at birth.

The primary objective of this study was to estimate associations between maternal serum concentrations of PFAS and offspring birth weight and adiposity (percent fat mass) at birth. The secondary objectives were to estimate cross-sectional associations between maternal PFAS concentrations and fasting glucose and lipids at mid-pregnancy. Because the pathway by which prenatal PFAS exposure may lead to reduced body mass at birth has not been elucidated, we then investigated whether concentrations of certain maternal circulating nutrients mediate associations between PFAS concentrations and offspring weight and adiposity at birth.

Methods

Study Design and Participants

Healthy Start is a prospective cohort study that enrolled 1,410 pregnant women from obstetrics clinics at the University of Colorado Hospital from 2009–2014. Enrolled women were ≥16 years old with singleton pregnancies; no history of previous stillbirth or extremely preterm birth (<25 wk of gestation); no self-reported diagnosis of diabetes, cancer, asthma managed with steroids, or psychiatric illness; and <24 wk of gestation at enrollment. Participants completed two study visits during pregnancy and one at delivery. Study procedures were approved by the Colorado Multiple Institutional Review Board. Written informed consent was obtained from participants prior to the first study visit. The analysis of blinded specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

For this analysis, we used data from a convenience sample of 652 participants selected for PFAS measurements based on availability of maternal serum collected at mid-pregnancy and umbilical cord blood at delivery. We excluded preterm births, who may have distinct determinants of adiposity (Uthaya et al. 2005), and participants missing exposure or outcome data.

Exposure Measurement

Fasting maternal blood samples were collected during pregnancy (median: 27th week of gestation; range: 20 to 34 wk), and serum was separated and stored at −80°C. Blood samples were collected between 2010 and 2014. Serum samples were shipped on dry ice to the CDC, where 11 PFAS were quantified using a modification of a previously published method (Kato et al. 2011a): perfluorooctane sulfonamide, 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate, 2-(*N*-methyl-perfluorooctane sulfonamido) acetate, perfluorohexane sulfonate (PFHxS), n-PFOA, sum of branched isomers of PFOA (Sb-PFOA), perfluorodecanoate (PFDeA), n-perfluorooctane sulfonate (n-PFOS), sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS), sum of perfluorodimethylhexane sulfonate isomers (Sm2-PFOS), and perfluorononanoate (PFNA). Analytical standards, quality control (QC) materials, and reagent blank samples were included in each batch along with study samples, and QC concentrations in each batch were evaluated using standard statistical probability rules. For most analytes, coefficients of variation in a 4-month period were <10%; higher values (up to 14.9%) were obtained for the three branched isomers of PFOA and PFOS at concentrations ranging from 0.3 to 1.5 ng/mL. Branched and linear isomers of PFOS (n-PFOS, Sm-PFOS, Sm2-PFOS) and PFOA (n-PFOA, Sb-PFOA) were summed to produce total concentrations of PFOS and PFOA, respectively. The limit of detection (LOD) for all PFAS was 0.1 ng/ml. For concentrations below the LOD, we used the instrument-reported values. Values of zero were replaced by the minimum reported concentration divided by 2 to allow log transformation. PFAS detectable in >50% of participants were included in this analysis.

Outcome Measurement

Birth weight was measured by clinical personnel at birth using a calibrated scale. Neonatal body composition was measured within 3 days of birth using the PeaPod device (COSMED). The PeaPod uses air displacement plethysmography to estimate fat mass and fat-free mass (e.g., bone, water, nonbone mineral, and protein) (Urlando et al. 2003). Adiposity (percent fat mass) estimates from this two-compartment model have been shown to be highly reproducible and not to differ significantly from the reference four-compartment model (Ellis et al. 2007). Trained research personnel conducted at least two measurements of each infant using the PeaPod device; a third measurement was conducted if the adiposity estimates differed by >2% (26% of infants). The average of the closest two measures was used.

Other Variables

Maternal fasting plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were analyzed at the University of Colorado Hospital Clinical and Translational Research Center using an AU400e Chemistry Analyzer (Olympus America, Center Valley, PA, USA), using aliquots of the same blood draw used to measure PFAS. Non-HDL cholesterol was calculated in milligrams per deciliter by subtracting HDL cholesterol from total cholesterol.

Information on maternal age, race and ethnicity, education, household income, and number of previous pregnancies was obtained via questionnaires administered at the first in-person study visit, and current smoking was also reported at each subsequent visit. Maternal height was measured at the first study visit. Maternal weight prior to pregnancy was obtained from the medical record or, if unavailable, from self-report at the first study visit. Prepregnancy body mass index (BMI) was calculated as weight prior to pregnancy in kilograms divided by height in meters squared. Gestational weight gain was calculated by subtracting the prepregnancy weight from the last measured weight during pregnancy, obtained from the prenatal medical record. Infant sex and gestational age at birth were obtained from medical records.

Statistical Analysis

Univariate distributions of all exposures, outcomes, and covariates were examined to identify implausible values and assess normality. Concentrations of PFAS were categorized into tertiles for PFOA, PFOS, and PFHxS, and above vs. at or below the median for PFNA and PFDeA, due to limited variability. Continuous PFAS concentrations were natural log–transformed for analysis. Spearman correlation coefficients were estimated for each pair of PFAS. Distributions of glucose and triglycerides were right-skewed and were natural log–transformed, while total cholesterol, HDL cholesterol, and non-HDL cholesterol were normally distributed and were untransformed.

Separate linear regression models estimated associations of each PFAS exposure, as categories or as continuous natural log–transformed concentrations, with the following infant outcomes: birth weight, adiposity (percent fat mass) at birth, fat mass, and fat-free mass at birth. The following potential confounders were included *a priori* based on previously reported associations with birth weight or adiposity, and/or for comparability with previous studies (Darrow et al. 2013; Fei et al. 2007; Johnson et al. 2014; Starling et al. 2015; Whitworth et al. 2012): maternal age (years), prepregnancy BMI (kg/m^2), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, all others), educational level attained (less than high school, high school or equivalent, some college or associate's degree, four-year college, graduate degree), number of previous pregnancies (any vs. none), smoking during pregnancy (any vs. none), gestational weight gain (kg), infant sex, gestational age at birth (days), and gestational age at maternal blood draw (days). Statistical interactions between each PFAS and infant sex were evaluated by including a product term and removing it if nonsignificant ($p > 0.15$). Sensitivity analyses were conducted to examine the potential for confounding by correlated exposures in models for birth weight and adiposity by first including all five continuous, natural log–transformed PFAS in a multipollutant regression model fit by least squares, and second, fitting a penalized regression model on the same set of variables using elastic net regularization (Zou and Hastie 2005) with the degree of shrinkage determined by random 10-fold cross-validation. Elastic net models were fit using PROC GLMSELECT in SAS (version 9.4; SAS Institute Inc.)

Cross-sectional associations between maternal PFAS concentrations and maternal glucose and lipids were estimated using separate linear regression models for each nutrient. Covariates were the same as above, excluding infant sex, gestational age at birth, and gestational weight gain.

We considered for mediation analysis those PFAS associated with neonatal outcomes, and also with maternal circulating nutrients previously associated with those outcomes. We decomposed the total effect of maternal PFAS concentration on birth weight or adiposity into two components: the natural direct effect, which is the effect of PFAS concentration on the neonatal outcome that is not mediated through changes in maternal circulating nutrients, and the natural indirect effect, the effect that is mediated through changes in maternal circulating nutrients. We first tested for exposure–mediator interaction by evaluating PFAS-by-nutrient interaction terms in separate models for birth weight and adiposity. We then estimated the natural indirect effect, natural direct effect, and total effect using an imputation-based method implemented in the R package medflex (Steen et al. 2017; Vansteelandt et al. 2012). In the absence of exposure–mediator interaction, the total effect is equal to the sum of the natural direct effect and the natural indirect effect. We calculated the percent mediated as the natural indirect effect divided by the total effect $\times 100\%$ (VanderWeele 2016). Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc.) and R (version 3.1.3; R Foundation for Statistical Computing).

Results

Among 1,410 participants enrolled in the Healthy Start cohort study, 17 experienced fetal demise, 11 withdrew from the study, and 83 delivered preterm infants. Of the remaining 1,299 potentially eligible participants, 633 had PFAS concentrations measured in mid-pregnancy serum. For analyses with birth weight or glucose as dependent variables, we excluded five participants missing glucose measurements, leading to a sample size of 628 (Figure S1). For analyses with cholesterol or triglycerides as dependent variables, we excluded 30 participants missing lipid measurements, leading to a sample size of 598. For analyses of body composition, we excluded 24 infants without body composition measurements within 3 days of birth, leading to a sample size of 604. There was no significant difference in mean birth weight between infants with body composition measurements and those without (3,298 g vs. 3,267 g; $t = 0.35$, $p = 0.72$). There were no other missing data in exposures, outcomes, or covariates of interest.

The 628 participants did not differ meaningfully in any maternal characteristics from the larger Healthy Start study population of 1,299 with term births (Table S1). The mean $\pm$ standard deviation age at delivery was 27.8 ± 6.2 y, and the mean prepregnancy BMI was $25.8 \pm 6.5\ kg/m^2$ (Table 1). Approximately 44% of the women identified as race/ethnicity other than non-Hispanic white. Gestational diabetes was diagnosed in 27 women. Maternal characteristics, including parity and race/ethnicity, varied by tertiles of PFOA concentration.

Of the 11 PFAS measured, five were detected in >50% of participants after summing branched and linear isomers of PFOA and PFOS, respectively (Table S2). Concentrations were somewhat lower than the concentrations among a nationally representative sample of females 12 y old and older (Table 2). PFAS concentrations showed moderate to high pairwise correlations, with the greatest Spearman correlation between PFOA and PFNA ($\rho = 0.76$) (Table S3).

Certain PFAS were inversely associated with birth weight and adiposity. There were no significant interactions between any PFAS and infant sex in their associations with neonatal outcomes; therefore, estimates are presented for both sexes combined. In covariate-adjusted models, each ln-unit increase in PFOA and PFNA were associated with a reduction in birth weight (Table 3). Additionally, women in the highest categories of PFOA and PFNA concentrations had offspring with lower birth weight compared to the lowest categories. Birth weight was also lower in the highest tertile of PFOS concentration compared to the lowest tertile, although the difference did not reach statistical significance.

The highest categories of PFOA, PFNA, and PFHxS were associated with significantly reduced offspring adiposity, relative to the lowest categories. Offspring adiposity was also somewhat lower for each ln-unit increase in continuous PFOA and PFHxS, although estimates were imprecise, and confidence limits included the null. PFOA and PFNA (as continuous variables) were inversely associated with fat mass (Table S4). The highest categories of PFOA, PFNA, and PFHxS were associated with reduced fat mass vs. the lowest categories. PFOS and PFDeA were not associated with birth weight or adiposity. There were no associations between PFAS and fat-free mass.

Certain maternal nutrients were cross-sectionally associated with PFAS concentrations. The highest categories of PFOA, PFNA, PFDeA, and PFHxS were associated with reduced glucose, vs. the lowest categories (Table 4). PFOS was not associated with glucose. Continuous PFOA, PFNA, and PFDeA were also inversely associated with glucose, such that a log-unit increase in these PFAS was associated with an approximately 2% to 3% reduction in glucose. Continuous PFOA and PFHxS were associated with higher HDL cholesterol (Table S5). The highest tertile of PFHxS was associated with lower ln-triglycerides vs. the lowest tertile. None of the PFAS were associated with total cholesterol or non-HDL cholesterol (not shown).

We evaluated the potential for maternal circulating nutrients to mediate the effect of PFAS on offspring weight and adiposity by first examining pairwise associations between each PFAS and nutrient (described above), and between each nutrient and

Table 1. Characteristics of 628 eligible mother–infant pairs in Healthy Start, 2009–2014, and by tertile of maternal serum perfluorooctanoate concentration (ng/ml).

Study group variables	All participants	Tertile 1 (0.1–0.8)	Tertile 2 (0.9–1.4)	Tertile 3 (1.4–17.0)
Age at delivery (years)	27.8 ± 6.2	28.0 ± 6.3	26.9 ± 6.5	28.5 ± 5.6
16–19	77 (12)	20 (9)	39 (19)	18 (9)
20–24	127 (20)	59 (26)	39 (19)	29 (14)
25–29	152 (24)	46 (21)	44 (22)	62 (31)
30–34	186 (30)	62 (28)	56 (27)	68 (34)
35–39	75 (12)	32 (14)	22 (11)	21 (10)
40–45	11 (2)	5 (2)	4 (2)	2 (1)
Prepregnancy body mass index (kg/m^2)	25.8 ± 6.5	25.9 ± 6.1	26.3 ± 6.8	25.2 ± 6.7
<18.5	18 (3)	6 (3)	8 (4)	4 (2)
18.5–24.9	323 (51)	112 (50)	90 (44)	121 (60)
25.0–29.9	167 (27)	56 (25)	63 (31)	48 (24)
≥30.0	120 (19)	50 (22)	43 (21)	27 (14)
Race/ethnicity *n* (%)				
Non-Hispanic white	349 (56)	97 (43)	104 (51)	148 (74)
Hispanic	144 (23)	63 (28)	58 (28)	23 (12)
Non-Hispanic black	95 (15)	53 (24)	27 (13)	15 (8)
All others	40 (6)	11 (5)	15 (7)	14 (7)
Education *n* (%)				
Less than 12th grade	89 (14)	40 (18)	34 (17)	15 (8)
High school degree or equivalent	111 (18)	46 (21)	34 (17)	31 (16)
Some college or associate's	139 (22)	52 (23)	56 (27)	31 (16)
Four-year college (bachelor's)	138 (22)	41 (18)	38 (19)	59 (30)
Graduate degree	151 (24)	45 (20)	42 (21)	64 (32)
Household income *n* (%)				
$20,000 or less	88 (14)	43 (19)	31 (15)	14 (7)
$20,001 to $40,000	80 (13)	29 (13)	33 (16)	18 (9)
$40,001 to $70,000	124 (20)	43 (19)	37 (18)	44 (22)
$70,001 or more	217 (35)	56 (25)	69 (34)	92 (46)
Don't know	119 (19)	53 (24)	34 (17)	32 (16)
Any previous pregnancies *n* (%)	395 (63)	182 (81)	121 (59)	92 (46)
Any smoking during pregnancy *n* (%)	55 (9)	22 (10)	16 (8)	17 (8)
Gestational weight gain (kg)	14.1 ± 6.3	13.5 ± 6.8	14.0 ± 6.2	14.8 ± 5.9
Fasting glucose (mg/dl)	78.4 ± 8.8	79.4 ± 8.4	78.5 ± 10.2	77.0 ± 7.6
Fasting total cholesterol (mg/dl)[a]	212.7 ± 39.6	209.5 ± 41.2	213.4 ± 39.8	215.2 ± 37.7
Fasting HDL cholesterol (mg/dl)[a]	63.8 ± 13.4	62.2 ± 12.9	64.1 ± 12.7	65.3 ± 14.4
Fasting non-HDL cholesterol (mg/dl)[a]	148.8 ± 36.0	147.3 ± 37.7	149.3 ± 35.9	149.9 ± 34.5
Fasting triglycerides (mg/dl)[a]	161.6 ± 59.4	159.6 ± 52.2	167.4 ± 66.9	157.8 ± 58.3
Cesarean delivery *n* (%)	132 (21)	41 (18)	48 (24)	43 (22)
Female infant *n* (%)	299 (48)	103 (46)	92 (45)	104 (52)
Gestational age at birth (days)	277 ± 8	276 ± 7	277 ± 8	279 ± 8
Infant birth weight (g)	3,297 ± 420	3,300 ± 448	3,319 ± 425	3,271 ± 383
Infant adiposity at birth (% fat mass)[b]	9.1 ± 3.8	9.3 ± 4.0	9.1 ± 3.9	8.8 ± 3.5

Note: Data are shown as Mean ± SD or *n* (%). HDL, high-density lipoprotein; SD, standard deviation.
[a]30 participants were missing lipid measures during pregnancy.
[b]24 participants were missing infant adiposity measures at birth.

neonatal outcomes. Glucose was the only nutrient associated with PFAS and also associated with fat mass and adiposity in this cohort, as reported previously (Crume et al. 2015). Therefore, associations between continuous measures of PFOA, PFNA, and birth weight and associations between categories of PFOA, PFNA, PFHxS, and adiposity were examined for potential mediation by glucose. None of the interaction terms for PFOA, PFNA, or PFHxS with glucose were significant at $p < 0.15$.

Results of the mediation analysis of the association between continuous PFOA and birth weight indicated that 3.0% of the total effect was mediated through maternal glucose concentrations (percent mediated calculated as natural indirect effect divided by total effect ×100%). Similarly, 2.5%

Table 2. Serum concentrations of perfluoroalkyl substances (ng/ml) among 628 eligible participants in Healthy Start, 2009–2014.

		This study								
				Percentiles						
Compound	Abbreviation	Percent detected	LOD	5th	25th	50th	75th	95th	Geometric mean (95% CI)	Geometric mean (95% CI) among NHANES females 2011–2012[a]
Perfluorooctanoate[b]	PFOA	100	0.1	0.3	0.7	1.1	1.6	2.7	1.04 (0.99, 1.10)	1.84 (1.68, 2.01)
Perfluorooctane sulfonate[b]	PFOS	99	0.1	0.7	1.5	2.4	3.7	6.7	2.30 (2.17, 2.43)	5.10 (4.70, 5.53)
Perfluorononanoate	PFNA	98	0.1	0.2	0.3	0.4	0.6	1.1	0.39 (0.38, 0.41)	0.824 (0.762, 0.890)
Perfluorodecanoate	PFDeA	65	0.1	<LOD	<LOD	0.1	0.2	0.4	0.14 (0.13, 0.14)	0.193 (0.177, 0.211)
Perfluorohexane sulfonate	PFHxS	99	0.1	0.2	0.5	0.8	1.2	2.8	0.75 (0.70, 0.80)	0.989 (0.876, 1.12)

Note: CI, confidence interval; LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey.
[a]CDC, Fourth National Report on Human Exposure to Environmental Chemicals, updated tables, February 2015 (www.cdc.gov/exposurereport).
[b]PFOA and PFOS measures are the sum of linear and branched isomers.

Table 3. Maternal serum perfluoroalkyl substances and offspring weight (n=628) and adiposity (n=604) at birth in Healthy Start, 2009–2014.

Concentration	Unadjusted change in birth weight (g) and 95% CI	Adjusted[a] change in birth weight (g) and 95% CI	Unadjusted change in adiposity (% fat mass) and 95% CI	Adjusted[a] change in adiposity (% fat mass) and 95% CI
PFOA (ng/ml)[b]	−9.9 (−59.7, 39.8)	−51.4 (−97.2, −5.7)	−0.26 (−0.72, 0.19)	−0.43 (−0.91, 0.04)
0.1–0.8	Reference	Reference	Reference	Reference
0.9–1.4	18.7 (−61.2, 98.7)	−15.9 (−84.9, 53.2)	−0.20 (−0.94, 0.54)	−0.34 (−1.06, 0.38)
1.4–17.0	−29.5 (−109.9, 50.9)	−92.4 (−166.2, −18.5)	−0.56 (−1.30, 0.18)	−0.97 (−1.74, −0.20)
PFOS (ng/ml)[b]	1.7 (−43.2, 46.7)	−13.8 (−53.8, 26.3)	0.07 (−0.34, 0.48)	0.08 (−0.33, 0.49)
<LOD−1.8	Reference	Reference	Reference	Reference
1.8–3.2	−2.5 (−83.8, 78.9)	−33.8 (−102.8, 35.2)	0.30 (−0.46, 1.05)	0.26 (−0.46, 0.98)
3.2–15.6	−43.9 (−124.0, 36.3)	−71.1 (−142.6, 0.5)	−0.28 (−1.01, 0.46)	−0.41 (−1.15, 0.33)
PFNA (ng/ml)[b]	−8.4 (−61.3, 44.5)	−57.6 (−104.1, −11.2)	−0.30 (−0.78, 0.18)	−0.38 (−0.86, 0.11)
<LOD−0.4	Reference	Reference	Reference	Reference
0.5–6.0	−41.0 (−108.1, 26.1)	−92.1 (−150.6, −33.6)	−0.76 (−1.38, −0.14)	−0.85 (−1.46, −0.24)
PFDeA (ng/ml)[b]	25.7 (−30.4, 81.9)	11.5 (−37.3, 60.4)	0.03 (−0.49, 0.55)	0.06 (−0.45, 0.56)
≤0.1	Reference	Reference	Reference	Reference
0.2–3.5	16.8 (−50.8, 84.5)	0.4 (−58.4, 59.1)	−0.13 (−0.76, 0.49)	−0.16 (−0.77, 0.45)
PFHxS (ng/ml)[b]	8.3 (−32.5, 49.1)	−13.5 (−50.7, 23.7)	−0.26 (−0.64, 0.11)	−0.32 (−0.70, 0.07)
<LOD−0.5	Reference	Reference	Reference	Reference
0.6–1.0	78.0 (−1.7, 157.7)	32.9 (−36.3, 102.0)	0.35 (−0.38, 1.08)	0.12 (−0.59, 0.83)
1.1–10.9	6.5 (−74.5, 87.6)	−31.8 (−105.8, 42.2)	−0.87 (−1.61, −0.12)	−0.99 (−1.75, −0.23)

Note: CI, confidence interval; PFDeA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; LOD, limit of detection. LOD was 0.1 ng/ml for all PFAS.
[a]Adjusted for maternal age, prepregnancy body mass index (BMI), race/ethnicity, education, gestational weight gain, smoking during pregnancy, gravidity, gestational age at blood draw, infant sex, and gestational age at birth.
[b]Beta coefficients per natural log unit increase in each perfluoroalkyl substance, and for upper categories compared to the lowest. PFOA and PFOS measures are the sum of linear and branched isomers.

of the effect of continuous PFNA on birth weight was mediated by maternal glucose concentrations. Mediation analysis of the associations between categories of selected PFAS and adiposity indicated that 9.2% of the effect of PFOA, 11.6% of the effect of PFNA, and 10.4% of the effect of PFHxS on adiposity were mediated by maternal glucose concentrations (Table S6).

A sensitivity analysis was performed to evaluate the potential for confounding by other correlated PFAS in single-exposure models. In a multipollutant model for birth weight fit by least squares, only continuous PFNA had a significant inverse association with birth weight; continuous PFOA was also associated with lower birth weight, but the estimate was not statistically significantly different from the null. The imprecision of this estimate may be related to the fact that the variance inflation factor for PFOA was above 3, indicating possible multicollinearity (Table S7). In the same model fit by elastic net, allowing simultaneous variable selection and estimation, PFOA and PFNA were both inversely associated with birth weight, and PFDeA was positively associated with birth weight. In a multipollutant model for adiposity fit by least squares, PFOA, PFNA, and PFHxS showed nonsignificant inverse associations with adiposity, and PFOS was positively associated. In the same model fit by elastic net, PFOA, PFNA, and PFHxS were inversely associated with adiposity, while PFOS remained positively associated. Although penalized regression estimates are known to be biased, these results are presented as complementary to the single exposure models, in consideration of the fact that PFAS exposure occurs as mixtures rather than as single pollutants.

Discussion

In an ethnically diverse sample of mother–infant pairs, we found inverse associations of certain maternal serum PFAS concentrations

Table 4. Maternal serum perfluoroalkyl substances and fasting glucose among 628 eligible participants in Healthy Start, 2009–2014.

Concentration	Unadjusted change in ln-glucose and 95% CI	Adjusted[a] change in ln-glucose and 95% CI
PFOA (ng/ml)[b]	−0.021 (−0.033, −0.008)	−0.018 (−0.031, −0.005)
0.1–0.8	Reference	Reference
0.9–1.4	−0.012 (−0.032, 0.008)	−0.014 (−0.034, 0.006)
1.4–17.0	−0.029 (−0.049, −0.009)	−0.025 (−0.046, −0.004)
PFOS (ng/ml)[b]	−0.017 (−0.028, −0.006)	−0.009 (−0.020, 0.003)
<LOD−1.8	Reference	Reference
1.8–3.2	0.005 (−0.015, 0.025)	0.011 (−0.009, 0.030)
3.2–15.6	−0.021 (−0.041, −0.001)	−0.009 (−0.029, 0.011)
PFNA (ng/ml)[b]	−0.019 (−0.032, −0.006)	−0.017 (−0.030, −0.004)
<LOD−0.4	Reference	Reference
0.5–6.0	−0.029 (−0.045, −0.012)	−0.025 (−0.042, −0.009)
PFDeA (ng/ml)[b]	−0.030 (−0.043, −0.016)	−0.027 (−0.041, −0.014)
≤0.1	Reference	Reference
0.2–3.5	−0.027 (−0.044, −0.011)	−0.024 (−0.041, −0.007)
PFHxS (ng/ml)[b]	−0.016 (−0.026, −0.006)	−0.011 (−0.021, 0.000)
<LOD−0.5	Reference	Reference
0.6–1.0	−0.011 (−0.031, 0.009)	−0.009 (−0.029, 0.010)
1.1–10.9	−0.034 (−0.055, −0.014)	−0.023 (−0.044, −0.002)

Note: Glucose modeled as a natural log–transformed variable. CI, confidence interval; PFDeA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; LOD, limit of detection. LOD was 0.1 ng/ml for all PFAS.
[a]Adjusted for maternal age, prepregnancy body mass index (BMI), race/ethnicity, education, smoking during pregnancy, gravidity, and gestational age at blood draw.
[b]Beta coefficients per natural log unit increase in each perfluoroalkyl substance, and for upper categories compared to the lowest. PFOA and PFOS measures are the sum of linear and branched isomers.

with offspring weight and adiposity at birth. We also observed inverse associations between some PFAS and maternal fasting glucose concentrations at mid-pregnancy, suggesting that reduced availability of maternal glucose reaching the fetus could be a potential pathway linking PFAS exposure to reduced infant weight and adiposity at birth. However, only a small proportion of the effect of maternal PFAS concentrations on offspring birth weight and adiposity was mediated through maternal glucose concentrations, if assumptions for causal interpretation are met.

Several recent epidemiologic studies have reported inverse associations between certain PFAS and birth weight (Bach et al. 2015b; Darrow et al. 2013; de Cock et al. 2016; Fei et al. 2007; Kishi et al. 2015; Maisonet et al. 2012; Wang et al. 2016; Whitworth et al. 2012), although not all estimates have been statistically significant. Two recent systematic reviews of epidemiologic evidence independently concluded that lower birth weight is associated with higher PFOA concentrations during pregnancy (Bach et al. 2015a; Johnson et al. 2014). One of these reviews employed the Navigation Guide methodology to evaluate the strength and quality of the evidence (Johnson et al. 2014), and results were used in an integrated assessment of evidence from animal and human studies to conclude that PFOA is "known to be toxic" to reproduction and development in humans (Lam et al. 2014). Our results are in agreement with previous findings of an inverse association between PFOA and birth weight, and provide additional evidence for an inverse association between PFNA and birth weight. While some previous studies reported effect modification by offspring sex (de Cock et al. 2016; Kishi et al. 2015; Wang et al. 2016), we found no evidence that associations between PFAS and birth weight or adiposity differed by sex.

To our knowledge, this is the first study reporting associations of maternal PFAS concentrations with offspring body composition at birth. We found that the highest categories of maternal PFOA, PFNA, and PFHxS were associated with lower offspring adiposity, corresponding to an approximate 10% reduction from the highest to lowest concentration categories, given the mean adiposity at birth of 9.1% fat mass in this population. Adiposity at birth is believed to be sensitive to adverse *in utero* exposures and may serve to predict future obesity risk more accurately than birth weight (Catalano et al. 2009).

Previous studies (Catalano et al. 2003; HAPO Study Cooperative Research Group 2009), including data from Healthy Start (Crume et al. 2015), have identified maternal fasting glucose during pregnancy as a predictor of offspring adiposity, independent of maternal prepregnancy BMI. We found inverse associations between multiple PFAS and maternal fasting glucose. We therefore explored the possibility that certain PFAS led to lower adiposity and weight at birth through the mediator of maternal glucose. The results of our mediation analysis suggest that a small percentage (2.5–3.0%) of the effect of PFAS on birth weight is mediated through maternal glucose. However, a slightly larger percentage (9.2–11.6%) of the effect of PFAS on adiposity may be mediated through reductions in maternal glucose. The complete mechanism by which prenatal PFAS exposure may lead to reduced offspring birth weight and adiposity remains unknown, but our results suggest that reduced maternal circulating glucose during pregnancy may explain part of this observed association.

Evidence from animal and *in vitro* studies supports the role of PFAS in altered glucose metabolism. In mice, exposure to PFOA produced reductions in blood glucose without changes in food intake (Ngo et al. 2014), greater insulin sensitivity and reduced hepatic glycogen synthesis (Yan et al. 2015), and changes in expression of genes associated with glucose metabolism (Abbott et al. 2012; Rosen et al. 2007). Observational studies in humans have produced inconsistent results. Three studies have examined PFAS concentrations and glucose-related outcomes in pregnant women. One study within a Canadian birth cohort with PFAS measured in early pregnancy reported no associations between PFOA or PFOS and impaired glucose tolerance or gestational diabetes, but elevated risk in the second quartile of PFHxS concentration only (Shapiro et al. 2016). Another study measured PFAS in U.S. women prior to conception and found positive associations with gestational diabetes for PFOA but none of the other six PFAS evaluated (Zhang et al. 2015). One other study among Chinese pregnant women reported a significant positive cross-sectional correlation with glucose for only one (perfluoroundecanoate) of the 17 PFAS examined; no covariate adjustment was performed (Jiang et al. 2014).

Among nonpregnant individuals, one study using data from the U.S. National Health and Nutrition Examination Survey (NHANES) 1999–2000 and 2003–2004 reported that serum PFNA was associated with lower blood insulin and decreased beta cell function in adolescents, while PFOS and PFOA were associated with higher blood insulin and increased beta cell function in adults, with no overall association with blood glucose (Lin et al. 2009). A separate study using only 2003–2004 NHANES data found no overall associations between PFOS, PFOA, PFNA, or PFHxS and insulin resistance (HOMA-IR), although the upper quartiles of PFOA and PFHxS concentrations were associated with lower HOMA-IR in adolescent females only, and the highest quartile of PFNA was associated with greater HOMA-IR in adult females only (Nelson et al. 2010). In a study of nonpregnant adults in Taiwan, greater serum PFOA concentrations were associated with lower fasting blood glucose, while individuals in the highest quartile of PFOS concentrations had higher fasting blood glucose compared to those in the lowest quartile (Su et al. 2016). Finally, a study of Canadian nonpregnant adults reported no significant associations between PFOA, PFOS, or PFHxS and fasting glucose or insulin (Fisher et al. 2013). Associations between PFAS concentrations and glucose metabolism therefore may vary according to the specific PFAS studied, as well as potentially by differences in population characteristics (e.g., pregnant vs. nonpregnant, sex, age, race/ethnicity, and geographic differences) and in covariate adjustment.

Mediation results should be interpreted with caution, as strong assumptions are required to allow causal interpretation of these findings. Specifically, causal interpretation requires no unmeasured confounders of the association between the main exposure and outcome, and also no unmeasured confounders of the exposure–mediator association and of the mediator–outcome association (VanderWeele 2016). While we can never be certain these conditions are met in an observational study, we have adjusted for known strong (nongenetic) predictors of maternal glucose and of birth weight and body composition, including maternal prepregnancy BMI, gravidity, gestational weight gain, smoking, infant sex, and gestational age at birth. There may be shared genetic influences on maternal circulating nutrients and offspring weight and body composition at birth; however, we do not expect these factors to be strongly associated with PFAS concentrations, so they are unlikely to act as exposure–mediator or exposure–outcome confounders.

Our findings on the associations of PFAS with maternal nutrients other than glucose were more equivocal. The highest tertile of PFHxS, but no other PFAS, was inversely associated with triglycerides. A recent study in Japan examining PFOS and PFOA reported an inverse association of PFOS with triglycerides during pregnancy (Kishi et al. 2015). The study in Japan analyzed

samples collected in 2002–2005 and reported a median PFOS concentration of 5.6 ng/ml, while our samples were collected in 2009–2014 and had a median PFOS concentration of 2.4 ng/ml. In contrast with some previous studies among pregnant women (Skuladottir et al. 2015; Starling et al. 2014), we found no association between PFAS concentrations and total cholesterol. Concentrations of certain PFAS, particularly PFOS, have generally declined over the last decade in the United States and elsewhere (Glynn et al. 2012; Kato et al. 2011b; Toms et al. 2014). It is therefore possible that PFOS may be associated with the outcomes studied at concentrations higher than we observed. All PFAS concentrations in our study were somewhat lower than those of females from the U.S. general population. The reasons for this difference are not obvious, but it is notable that the U.S. mean values also include regions with historical PFAS production where the general population was exposed via industrial emissions and contamination of drinking water (Braun et al. 2016; Pinney et al. 2014; Steenland et al. 2009). It remains difficult to predict where populations may experience such exposure, in part due to the fact that reporting of PFAS emissions have not been required on the U.S. Environmental Protection Agency's Toxics Release Inventory (U.S. EPA 2016).

We performed a sensitivity analysis to examine the potential for confounding by other correlated PFAS in single-exposure models. All five PFAS were included in multipollutant regression models fit by least squares for each of the primary outcomes: birth weight and adiposity at birth. Given the moderate to high pairwise correlations between PFAS concentrations, we expected that these estimates would be potentially unreliable due to collinearity of predictors. We therefore also fit elastic net shrinkage models to provide greater stability and to identify which of the set of PFAS were most important predictors of each outcome. While shrinkage approaches produce biased estimates, they may be useful for selecting important variables from a set of correlated predictors (Greenland 2008; Sun et al. 2013). The multipollutant models fit by elastic net supported the inverse associations between PFOA and PFNA and birth weight, and between PFOA, PFNA, and PFHxS and adiposity at birth. These models also indicated potential positive associations between PFDeA and birth weight, and between PFOS and adiposity at birth. The results for PFDeA should be interpreted cautiously, given the limited variability of PFDeA in our population; however, the association with PFOS may merit further investigation and replication.

We used a single serum measurement of PFAS to estimate exposure. Given the relatively long half-lives of PFAS documented in nonpregnant individuals (e.g., 3.5 years for PFOA) (Olsen et al. 2007), a single measurement is likely to provide a reasonable estimate of long-term exposure. Other potential limitations of this study include restriction to term births, so results may not be generalizable to preterm infants. Finally, due to the number of statistical analyses performed, some results may have attained significance due to chance. Strengths of the study include the relatively large sample size from an ethnically diverse population and high quality measures of adiposity at birth.

The magnitudes of the associations between prenatal PFAS exposure and offspring birth weight and adiposity may be considerable at the population level. Small-for-gestational-age birth weight is associated with adverse cognitive, behavioral, and metabolic health outcomes (de Bie et al. 2010; Heinonen et al. 2010; Saenger et al. 2007). Infants with lower weight at birth may experience rapid "catch-up growth" in the first year of life, and rapid growth in infancy is associated with greater BMI in childhood and adulthood (Bjerregaard et al. 2014; Ong et al. 2000). Therefore, an inverse association between prenatal PFAS exposure and birth weight and adiposity does not rule out the possibility that PFAS may act as obesogens in humans.

Some recent studies have reported positive associations between prenatal exposure to PFAS, particularly PFOA, and offspring weight or adiposity in childhood or early adulthood (Braun et al. 2016; Halldorsson et al. 2012; Høyer et al. 2015; Mora et al. 2017). Some of these studies reported stronger associations in female offspring; for example, Halldorsson et al. (2012) reported greater risk of overweight and obesity in females but not males born to mothers with higher prenatal concentrations of PFOA, Mora et al. (2017) found that higher prenatal concentrations of PFAS were associated with multiple measures of adiposity in mid-childhood in girls only, and Høyer et al. (2015) found associations between prenatal PFOA and PFOS and offspring waist-to-height ratio at age 5–9 years old that were stronger in girls than in boys. By contrast, Braun et al. (2016) found higher adiposity at age 8 among offspring of women in the second tertile of PFOA concentrations compared to the first, and effects were not modified by offspring sex. While we observed no sex-specific associations at birth, it is possible that male and female infants experience different growth patterns in early life following prenatal PFAS exposure. In support of the hypothesis that smaller size at birth may be followed by rapid growth, a study of British girls showed that the offspring of mothers with greater prenatal PFOS concentrations had lower weight at birth but greater weight at 20 months, after adjustment for birth weight (Maisonet et al. 2012).

Conclusions

Prenatal exposures to certain PFAS were inversely associated with offspring weight and adiposity at birth in an ethnically diverse convenience sample from a Colorado prospective cohort. Some PFAS were inversely associated with maternal fasting glucose at midpregnancy; however, only a small proportion of the effect of PFAS concentration on birth weight and adiposity appeared to be mediated through maternal glucose. Because both large and small birth size have been associated with increased risk of obesity and metabolic disease later in life, follow-up of offspring would be needed to determine the potential long-term effects of prenatal PFAS exposure on chronic disease risk in the general population.

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References

Abbott BD, Wood CR, Watkins AM, Tatum-Gibbs K, Das KP, Lau C. 2012. Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. Reprod Toxicol 33(4):491–505, PMID: 22154759, https://doi.org/10.1016/j.reprotox.2011.11.005.

Andersen CS, Fei C, Gamborg M, Nohr EA, Sørensen TI, Olsen J. 2013. Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. Am J Epidemiol 178(6):921–927.

Aylward LL, Hays SM, Kirman CR, Marchitti SA, Kenneke JF, English C, et al. 2014. Relationships of chemical concentrations in maternal and cord blood: A review of available data. J Toxicol Environ Health B Crit Rev 17(3):175–203, https://doi.org/10.1080/10937404.2014.884956.

Bach CC, Bech BH, Brix N, Nohr EA, Bonde JP, Henriksen TB. 2015a. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review. Crit Rev Toxicol 45(1):53–67, https://doi.org/10.3109/10408444.2014.952400.

Bach CC, Bech BH, Nohr EA, Olsen J, Matthiesen NB, Bonefeld-Jørgensen EC, et al. 2015b. Perfluoroalkyl acids in maternal serum and indices of fetal growth: The Aarhus birth cohort. Environ Health Perspect 124(6):848–854, https://doi.org/10.1289/ehp.1510046.

Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. 2005. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 331(7522):929, PMID: 16227306, https://doi.org/10.1136/bmj.38586.411273.E0.

Barry V, Winquist A, Steenland K. 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect 121(11-12):1313–1318, PMID: 24007715, https://doi.org/10.1289/ehp.1306615.

Bjerregaard LG, Rasmussen KM, Michaelsen KF, Skytthe A, Mortensen EL, Baker JL, et al. 2014. Effects of body size and change in body size from infancy through childhood on body mass index in adulthood. Int J Obes (Lond) 38 (10):1305–1311, PMID: 24942870, https://doi.org/10.1038/ijo.2014.108.

Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, et al. 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME Study. Obesity (Silver Spring) 24(1):231–237, PMID: 26554535, https://doi.org/10.1002/oby.21258.

Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, et al. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integr Environ Assess Manag 7(4):513–541, PMID: 21793199, https://doi.org/10.1002/ieam.258.

Cariou R, Veyrand B, Yamada A, Berrebi A, Zalko D, Durand S, et al. 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environ Int 84:71–81, PMID: 26232143, https://doi.org/10.1016/j.envint.2015.07.014.

Catalano PM, Thomas A, Huston-Presley L, Amini SB. 2003. Increased fetal adiposity: A very sensitive marker of abnormal in utero development. Am J Obstet Gynecol 189(6):1698–1704, PMID: 14710101.

Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, de Mouzon SH, et al. 2009. Perinatal risk factors for childhood obesity and metabolic dysregulation. Am J Clin Nutr 90(5):1303–1313, PMID: 19759171, https://doi.org/10.3945/ajcn.2008.27416.

CDC (Centers for Disease Control and Prevention). 2015. *Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2015*. Atlanta, GA:U.S. Department of Health and Human Services. http://www.cdc.gov/exposurereport/ [accessed 28 September 2016].

Claris O, Beltrand J, Levy-Marchal C. 2010. Consequences of intrauterine growth and early neonatal catch-up growth. Semin Perinatol 34(3):207–210, PMID: 20494737, https://doi.org/10.1053/j.semperi.2010.02.005.

Crume TL, Shapiro AL, Brinton JT, Glueck DH, Martinez M, Kohn M, et al. 2015. Maternal fuels and metabolic measures during pregnancy and neonatal body composition: the healthy start study. J Clin Endocrinol Metab 100(4):1672–1680, PMID: 25574704, https://doi.org/10.1210/jc.2014-2949.

Darrow LA, Stein CR, Steenland K. 2013. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environ Health Perspect 121(10):1207–1213, https://doi.org/10.1289/ehp.1206372.

de Bie HM, Oostrom KJ, Delemarre-van de Waal HA. 2010. Brain development, intelligence and cognitive outcome in children born small for gestational age. Horm Res Paediatr 73(1):6–14, PMID: 20190535, https://doi.org/10.1159/000271911.

de Cock M, De Boer MR, Lamoree M, Legler J, Van De Bor M. 2016. Prenatal exposure to endocrine disrupting chemicals and birth weight-A prospective cohort study. J Environ Sci Health A Tox Hazard Subst Environ Eng 51 (2):178–185.

Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC. 2007. Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. Am J Clin Nutr 85(1):90–95, PMID: 17209182.

Fei C, McLaughlin JK, Tarone RE, Olsen J. 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect 115(11):1677–1682, PMID: 18008003, https://doi.org/10.1289/ehp.10506.

Fisher M, Arbuckle TE, Wade M, Haines DA. 2013. Do perfluoroalkyl substances affect metabolic function and plasma lipids? Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res 121:95–103, PMID: 23266098, https://doi.org/10.1016/j.envres.2012.11.006.

Freinkel N. 1980. Banting lecture 1980. Of pregnancy and progeny. Diabetes 29 (12):1023–1035, PMID: 7002669.

Glynn A, Berger U, Bignert A, Ullah S, Aune M, Lignell S, et al. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environ Sci Technol 46(16):9071–9079, https://doi.org/10.1021/es301168c.

Greenland S. 2008. Invited commentary: Variable selection versus shrinkage in the control of multiple confounders. Am J Epidemiol 167(5):523–529; discussion 530–531.

Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, et al. 2012. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: A prospective cohort study. Environ Health Perspect 120(5):668–673, PMID: 22306490, https://doi.org/10.1289/ehp.1104034.

HAPO Study Cooperative Research Group, 2009. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes 58(2):453–459, https://doi.org/10.2337/db08-1112.

Heinonen K, Räikkönen K, Pesonen AK, Andersson S, Kajantie E, Eriksson JG, et al. 2010. Behavioural symptoms of attention deficit/hyperactivity disorder in preterm and term children born small and appropriate for gestational age: a longitudinal study. BMC Pediatr 10:91., PMID: 21159164, https://doi.org/10.1186/1471-2431-10-91.

Høyer BB, Ramlau-Hansen CH, Vrijheid M, Valvi D, Pedersen HS, Zviezdai V, et al. 2015. Anthropometry in 5- to 9-year-old Greenlandic and Ukrainian children in relation to prenatal exposure to perfluorinated alkyl substances. Environ Health Perspect 123(8):841–846, PMID: 25809098, https://doi.org/10.1289/ehp.1408881.

Jansson T, Powell TL. 2013. Role of placental nutrient sensing in developmental programming. Clin Obstet Gynecol 56(3):591–601, PMID: 23703224, https://doi.org/10.1097/GRF.0b013e3182993a2e.

Jiang W, Zhang Y, Zhu L, Deng J. 2014. Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environ Int 64:40–47, PMID: 24361923, https://doi.org/10.1016/j.envint.2013.12.001.

Johnson PI, Sutton P, Atchley DS, Koustas E, Lam J, Sen S, et al. 2014. The navigation guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect 122(10):1028–1039, PMID: 24968388, https://doi.org/10.1289/ehp.1307893.

Kato K, Basden BJ, Needham LL, Calafat AM. 2011a. Improved selectivity for the analysis of maternal serum and cord serum for polyfluoroalkyl chemicals. J Chromatogr A 1218(15):2133–2137, PMID: 21084089, https://doi.org/10.1016/j.chroma.2010.10.051.

Kato K, Wong LY, Jia LT, Kuklenyik Z, Calafat AM. 2011b. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. Environ Sci Technol 45(19):8307–8045, https://doi.org/10.1021/es1043613.

Kato K, Wong LY, Chen A, Dunbar C, Webster GM, Lanphear BP, et al. 2014. Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environ Sci Technol 48(16):9600–9608, https://doi.org/10.1021/es501811k.

Kishi R, Nakajima T, Goudarzi H, Kobayashi S, Sasaki S, Okada E, et al. 2015. The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: the Hokkaido study. Environ Health Perspect 123 (10):1038–1045, PMID: 25840032, https://doi.org/10.1289/ehp.1408834.

Lam J, Koustas E, Sutton P, Johnson PI, Atchley DS, Sen S, et al. 2014. The navigation guide-evidence-based medicine meets environmental health: integration of animal and human evidence for pfoa effects on fetal growth. Environ Health Perspect 122(10):1040–1051, PMID: 24968389, https://doi.org/10.1289/ehp.1307923.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 99(2):366–394, PMID: 17519394, https://doi.org/10.1093/toxsci/kfm128.

Lin CY, Chen PC, Lin YC, Lin LY. 2009. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32(4):702–707, PMID: 19114613, https://doi.org/10.2337/dc08-1816.

Maisonet M, Terrell ML, McGeehin MA, Christensen KY, Holmes A, Calafat AM, et al. 2012. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect 120(10):1432–1437, PMID: 22935244, https://doi.org/10.1289/ehp.1003096.

Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Basterrechea M, Grimalt JO, et al. 2015. Transfer of perfluoroalkyl substances from mother to fetus in a Spanish birth cohort. Environ Res 142:471–478, https://doi.org/10.1016/j.envres.2015.07.020.

Mora AM, Oken E, Rifas-Shiman SL, Webster TF, Gillman MW, Calafat AM, et al. 2017. Prenatal exposure to perfluoroalkyl substances and adiposity in early and mid-childhood. Environ Health Perspect 125(3)467–473, https://doi.org/10.1289/EHP246.

Nelson JW, Hatch EE, Webster TF. 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect 118(2):197–202, PMID: 20123614, https://doi.org/10.1289/ehp.0901165.

Ngo HT, Hetland RB, Sabaredzovic A, Haug LS, Steffensen IL. 2014. In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice. Environ Res 132:251–263.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115(9):1298–1305, PMID: 17805419, https://doi.org/10.1289/ehp.10009.

Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, et al. 2013. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int 60:89–96, PMID: 24013022, https://doi.org/10.1016/j.envint.2013.07.013.

Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. 2000. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 320(7240):967–971, PMID: 10753147.

Pinney SM, Biro FM, Windham GC, Herrick RL, Yaghjyan L, Calafat AM, et al. 2014. Serum biomarkers of polyfluoroalkyl compound exposure in young girls in Greater Cincinnati and the San Francisco Bay Area, USA. Environ Pollut 184:327–334, PMID: 24095703, https://doi.org/10.1016/j.envpol.2013.09.008.

Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. 2006. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 40(1):32–44, PMID: 16433330.

Rosen MB, Thibodeaux JR, Wood CR, Zehr RD, Schmid JE, Lau C. 2007. Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses. Toxicology 239(1-2):15–33, PMID: 17681415, https://doi.org/10.1016/j.tox.2007.06.095.

Saenger P, Czernichow P, Hughes I, Reiter EO. 2007. Small for gestational age: short stature and beyond. Endocr Rev 28(2):219–251, PMID: 17322454, https://doi.org/10.1210/er.2006-0039.

Secretariat of the Stockholm Convention. 2010. *The New POPs Under the Stockholm Convention*. Châtelaine, Switzerland:Stockholm Convention Clearing House. http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx [accessed 28 September 2016].

Shapiro GD, Dodds L, Arbuckle TE, Ashley-Martin J, Ettinger AS, Fisher M, et al. 2016. Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environ Res 147:71–81, PMID: 26852007, https://doi.org/10.1016/j.envres.2016.01.040.

Skuladottir M, Ramel A, Rytter D, Haug LS, Sabaredzovic A, Bech BH, et al. 2015. Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ Res 143:33–38, PMID: 26432473, https://doi.org/10.1016/j.envres.2015.09.001.

Starling AP, Engel SM, Whitworth KW, Richardson DB, Stuebe AM, Daniels JL, et al. 2014. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. Environ Int 62:104–112, PMID: 24189199, https://doi.org/10.1016/j.envint.2013.10.004.

Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, et al. 2015. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start Study. Am J Clin Nutr 101:302–309, PMID: 25646327, https://doi.org/10.3945/ajcn.114.094946.

Steen J, Loeys T, Moerkerke B, Vansteelandt S. 2017. medflex: an R package for flexible mediation analysis using natural effect models. J Stat Softw 76(11):1–46, https://doi.org/10.18637/jss.v076.i11.

Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am J Epidemiol 170(10):1268–1278, https://doi.org/10.1093/aje/kwp279.

Steenland K, Zhao L, Winquist A, Parks C. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environ Health Perspect 121(8):900–905, https://doi.org/10.1289/ehp.1206449.

Su TC, Kuo CC, Hwang JJ, Lien GW, Chen MF, Chen PC. 2016. Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environ Int 88:15–22, PMID: 26700417, https://doi.org/10.1016/j.envint.2015.11.016.

Sun Z, Tao Y, Li S, Ferguson KK, Meeker JD, Park SK, et al. 2013. Statistical strategies for constructing health risk models with multiple pollutants and their interactions: possible choices and comparisons. Environ Health 12(1):85., PMID: 24093917, https://doi.org/10.1186/1476-069X-12-85.

Toms LM, Thompson J, Rotander A, Hobson P, Calafat AM, Kato K, et al. 2014. Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011. Environ Int 71:74–80, https://doi.org/10.1016/j.envint.2014.05.019.

Urlando A, Dempster P, Aitkens S. 2003. A new air displacement plethysmograph for the measurement of body composition in infants. Pediatr Res 53(3):486–492, PMID: 12595599, https://doi.org/10.1203/01.PDR.0000049669.74793.E3.

U.S. EPA (U.S. Environmental Protection Agency). 2000. "EPA and 3M Announce Phase Out of PFOS." https://yosemite.epa.gov/opa/admpress.nsf/0/33aa946e6cb11f35852568e1005246b4 [accessed 28 September 2016].

U.S. EPA. 2016. "Toxics Release Inventory Program: TRI-Listed Chemicals." https://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals [accessed 28 September 2016].

Uthaya S, Thomas EL, Hamilton G, Doré CJ, Bell J, Modi N. 2005. Altered adiposity after extremely preterm birth. Pediatr Res 57(2):211–215, PMID: 15611357, https://doi.org/10.1203/01.PDR.0000148284.58934.1C.

VanderWeele TJ. 2016. Mediation analysis: a practitioner's guide. Annu Rev Public Health 37:17–32, PMID: 26653405, https://doi.org/10.1146/annurev-publhealth-032315-021402.

Vansteelandt S, Bekaert M, Lange T. 2012. Imputation Strategies for the Estimation of Natural Direct and Indirect Effects. Epidemiologic Methods 1(1):131–158, https://doi.org/10.1515/2161-962X.1014.

Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA, et al. 2016. Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan Maternal and Infant Cohort Study. Environ Health Perspect 124(11):1794–1800, PMID: 26895313, https://doi.org/10.1289/ehp.1509998.

Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, et al. 2012. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 175(12):1209–1216, PMID: 22517810, https://doi.org/10.1093/aje/kwr459.

Yan S, Zhang H, Zheng F, Sheng N, Guo X, Dai J. 2015. Perfluorooctanoic acid exposure for 28 days affects glucose homeostasis and induces insulin hypersensitivity in mice. Sci Rep 5:11029., PMID: 26066376, https://doi.org/10.1038/srep11029.

Zhang C, Sundaram R, Maisog J, Calafat AM, Barr DB, Buck Louis GM. 2015. A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. Fertil Steril 103(1):184–189, PMID: 25450302, https://doi.org/10.1016/j.fertnstert.2014.10.001.

Zou H, Hastie T. 2005. Regularization and variable selection via the elastic net. J Royal Statistical Soc B 67(2):301–320, https://doi.org/10.1111/j.1467-9868.2005.00503.x.